Received: 5 June 2019 | Revised: 18 July 2019 | Accepted: 21 July 2019

DOI: 10.1002/hbm.24745

RESEARCH ARTICLE

WILEY

Effects of hypogonadism on brain development during adolescence in girls with Turner syndrome

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Funding information
Fundamental Research Funds for the Central Universities; National Science Foundation of China, Grant/Award Numbers: 81671772, 91732101, 81701783; Research Fund of PLA of China, Grant/Award Number: AWS17J011

Abstract

Gonadal steroids play an important role in brain development, particularly during puberty. Girls with Turner syndrome (TS), a genetic disorder characterized by the absence of all or part of the second X chromosome, mostly present a loss of ovarian function and estrogen deficiency, as well as neuroanatomical abnormalities. However, few studies have attempted to isolate the indirect effects of hormones from the direct genetic effects of X chromosome insufficiency. Brain structural (i.e., gray matter [GM] morphology and white matter [WM] connectivity) and functional phenotypes (i.e., resting-state functional measures) were investigated in 23 adolescent girls with TS using multimodal MRI to assess the role of hypogonadism in brain development in TS. Specifically, all girls with TS were divided into a hormonally subnormal group and an abnormal subgroup according to their serum follicle-stimulating hormone (FSH) levels, with the karyotypes approximately matched between the two groups. Statistical analyses revealed significant effects of the "group-by-age" interaction on GM volume around the left medial orbitofrontal cortex and WM diffusion parameters around the bilateral corticospinal tract, anterior thalamic radiation, left superior longitudinal fasciculus, and cingulum bundle, but no significant "group-by-age" or group differences were observed in resting-state functional measures. Based on these findings, estrogen deficiency has a nontrivial impact on the development of the brain structure during adolescence in girls with TS. Our present study provides novel insights into the mechanism by which hypogonadism influences brain development during adolescence in girls with TS, and highlights the important role of estrogen replacement therapy in treating TS.

KEYWORDS
brain structural imaging, diffusion tensor imaging, gray matter volume, hypogonadism, the X chromosome, Turner syndrome, white matter connectivity

1 | INTRODUCTION

The brain is a major target of gonadal steroid hormones. Gonadal steroids putatively act on the brain in two different ways: (a) through organizational effects that permanently change the structure of the brain; and (b) through activation effects that temporarily change the functional activity of neural systems (McCarthy & Arnold, 2011). During puberty, the brain is particularly sensitive to gonadal steroids (Ahmed et al., 2008; Romeo, 2003; Schulz, Molenda-Figueira, & Sisk, 2009; Sisk & Zehr, 2005). The most notably hormonal changes during

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puberty are characterized by dramatically increased testosterone levels in boys and estradiol (E2) levels in girls. In addition to inducing the secondary sexual characteristics, these pubertal sex hormones are thought to play a critical role in refining brain maturation during puberty (Peper & Dahl, 2013).

Turner syndrome (TS) is a genetic disorder characterized by the absence of all or part of the second X chromosome and occurs in ~1/2500 live female births (Baena et al., 2004; Gravholt, 2005). The physical TS phenotypes include short stature and endocrine abnormalities, such as the loss of ovarian function and estrogen deficiency (Dhooge, De Vel, Verhoye, Lemmerling, & Vinck, 2005; Sybert & McCauley, 2004). Additionally, extensive evidence has revealed selective deficits in cognitive functions such as visuospatial reasoning, executive function, and social cognition in girls with TS (Garron, 1977; Hong & Reiss, 2014; Hong, Scaletta Kent, & Kesler, 2009; LaHood & Bacon, 1985; Zhao & Gong, 2017). Furthermore, neuroimaging studies have revealed both neuroanatomical and neurofunctional changes in patients with TS. For example, decreased gray matter volume (GMV) in parieto-occipital regions, amygdala, and hippocampus, as well as increased temporal GMV, were consistently reported in TS (Li et al., 2016; Marzelli, Hoeft, Hong, & Reiss, 2011; Molko et al., 2004; Reiss, Mazzocco, Greenlaw, Freund, & Ross, 1995). Also, TS patients showed impairment in white matter (WM) integrity, for example, decreased fractional anisotropy (FA) and increased mean diffusivity (MD) in widespread WM regions (Holzapfel, Barnea-Goraly, Eckert, Kesler, & Reiss, 2006; Yamagata et al., 2012), and disrupted WM organizational pattern of specific hemispheric modules (Zhao et al., 2019). In addition, resting-state functional connectivity strength was found to be significantly decreased in TS (Xie et al., 2017). However, these studies comparing TS with healthy controls have a limited capacity to disentangle the indirect effects of hormone deficiency from the direct genetic effects of X chromosome insufficiency (i.e., haploinsufficiency of gene products) on the nervous system (Zhao & Gong, 2017).

Notably, the absence of pubertal development is one of the most common clinical features of patients with TS, who should have experienced a sex hormone surge if the hypothalamic–pituitary-gonadal axis was activated normally. While up to 20% of girls with TS undergo some spontaneous pubertal development, few maintain normal ovarian function (Gravholt, 2004). Individual variations in hypogonadism across individuals with TS provide an opportunity to investigate the independent hormonal effects on girls with TS. To date, however, it remains unexplored whether and how the degree of hypogonadism influences brain development in patients with TS, which is important for understanding the effects of hormones on the brain and cognition in both patients with TS and healthy individuals.

In the current study, we aimed to examine the presence of a "hypogonadism effect" on brain development during adolescence in girls with TS. Therefore, adolescent patients with TS presenting with different degree of hypogonadism were compared in this study. A set of cognitive assessments was performed, and structural MRI, diffusion tensor imaging (DTI), and resting-state functional MRI (rs-fMRI) data were collected to evaluate brain morphology, WM integrity, and functional activity.

2 | METHODS

2.1 | Participants

Girls with TS whose serum levels of both follicle-stimulating hormone (FSH) and E2 had been measured were included in the present study (23 females; age range: 9.5–18.6 years). All patients with TS were recruited from the China-Japan Friendship Hospital (CJFH) and Peking Union Medical College Hospital (PUMCH), and the diagnoses were confirmed using a standard cytogenetic karyotype assessment of peripheral blood samples. Eight of the patients with TS had a nonmosaic 45XO karyotype (monosomy); 15 patients showed mosaicism with the 45XO karyotype in some cells and the full second X chromosome in other cells or showed other complex structural abnormalities in the X chromosome. All participants except one underwent growth hormone (GH) substitution, and four participants were on estrogen replacement (ER) therapy. At the time of MRI scanning, most participants were in Tanner stage 1 or 2, according to the breast and pubic hair development. The relevant clinical information for all participants (if available) were included in Table S1. The medical history of all participants was screened to ensure a lack of evidence for current or past major neurological or psychiatric disorders. Additionally, no visible abnormalities (e.g., WM hyperintensity) were observed on the MR images, which were examined by an experienced radiologist. Each participant was reimbursed for travel and accommodation expenses accrued when participating in this study. The research protocol was approved by the Research Ethics Committee of Beijing Normal University. Written informed consent was obtained from the legal guardian of each participant.

The serum FSH and E2 levels for each patient with TS were assessed twice. To investigate the effect of hypogonadism on the TS brain, all girls with TS were categorized using the diagnostic criterion of primary ovarian insufficiency (i.e., a basal FSH level greater than 30–40 mIU/mL). This diagnostic criterion was proposed by the Committee on Adolescent Health Care (American College of Obstetricians and Gynecologists, 2014). Accordingly, we used the FSH level of 40 mIU/mL as a cutoff and divided the 23 girls with TS into two groups: subnormal group (including TS girls with a blood serum FSH level < 40 mIU/mL; 9 subjects in total) and abnormal group (including TS girls with blood serum FSH level ≥ 40 mIU/mL; 14 subjects in total). Notably, there was no recommended E2 cutoff value for evaluating ovarian dysfunction in clinical practice, because it fluctuates dramatically during the menstrual cycle and is susceptible to other non-ovarian factors (e.g., body fat percentage and excessive exercise). Therefore, while the E2 level was also measured out together with the FSH for girls with TS, the subgrouping procedure did not involve any E2 cutoff. Here, the abnormal group was defined to include TS girls with very severe hypogonadism (reaching the level of primary ovarian insufficiency), and the subnormal group was defined to include the rest TS girls with mild hypogonadism or normal ovarian function.

2.2 | Cognitive assessment

Each participant performed cognitive assessments within 2 days before or after the MRI scan. The participants aged 6–16 years

(11 girls in the abnormal group and 7 girls in the subnormal group) were assessed with the Chinese version of the Wechsler Intelligence Scale for Children-Fourth Edition (WISC-IV). Five composite scores were generated using the WISC-IV: full scale intelligence quotient (FSIQ), verbal comprehension index (VCI), perceptual reasoning index (PRI), processing speed index (PSI), and working-memory index (WMI). For participants above 16 years, we did not apply assessments for their IQ scores.

2.3 | MRI acquisition

All MRI scans were obtained using a 3-T Siemens Tim Trio MRI scanner in the Imaging Center for Brain Research, Beijing Normal University. The head of each participant was secured using straps and foam pads to minimize head movement. High-resolution 3D T1-weighted images were sagittally acquired using a magnetization prepared rapid gradient echo (MPRAGE) sequence: 144 sagittal slices; echo time (TE), 3.39 ms; repetition time (TR), 2,530 ms; inversion time (TI), 1,100 ms; 1.33-mm slice thickness with no gap; acquisition matrix, 256 × 256; 1 × 1-mm in-plane resolution; and acquisition time, 8:07 min. Diffusion MRI was axially applied using a single-shot echo planar imaging-based sequence: coverage of the whole brain; 62 axial slices; TR, 8,000 ms; TE, 89 ms; one image without diffusion weighting (i.e., b = 0 s/mm^2), followed by 30 optimal nonlinear diffusion-weighted directions; average, 2; 2.2-mm slice thickness; acquisition matrix, 128 × 128; 2.2 × 2.2-mm in-plane resolution; and acquisition time, 9:08 min. During rs-fMRI scanning, all participants were instructed to relax with their eyes closed while remaining awake and not thinking systematically. Thirty-three axial slices covering the whole brain were acquired using the following echo-planar imaging sequence: TR, 2,000 ms; TE, 30 ms; flip angle, 90°; slice thickness/gap, 3.5/0.7 mm; acquisition matrix, 64 × 64; 3.1 × 3.1-mm in-plane resolution; a total of 200 volumes; and acquisition time, 6:44 min.

2.4 | MRI processing

2.4.1 | Measurements of the gray/white matter volume measures

Voxel-based morphometry (VBM) was performed on the structural T1-weighted images using the Computational Anatomy Toolbox (CAT12, http://dbm.neuro.uni-jena.de/cat/) embedded in the Statistical Parametric Mapping toolbox (SPM12, http://www.fil.ion.ucl.ac.uk/spm). Briefly, the T1 image of each subject was normalized to the Montreal Neurological Institute (MNI) template space and then segmented into GM, WM, and cerebrospinal fluid (CSF). Next, the segmented tissue components were modulated by scaling with the extent of changes in volume due to spatial registration to convert the voxel values of tissue concentration to volume measures. Finally, the normalized WMV and GMV maps were smoothed with an isotropic Gaussian kernel (full width at half maximum = 6 mm) before the statistical analyses.

2.4.2 | WM diffusion measures

Diffusion MRI images were processed with the PANDA pipeline toolbox (Cui, Zhong, Xu, He, & Gong, 2013). Briefly, PANDA uses the modules of the FMRIB Software Library (FSL 5.0.11) to complete the skull stripping, simple motion and eddy current corrections, diffusion tensor/parameter calculation, and spatial normalization (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012). For the analysis, the three most commonly used diffusion parameters, fractional anisotropy (FA), axial diffusivity (AD) and radial diffusivity (RD), were calculated. FA, AD, and RD represent the fractions of total diffusion that are attributed to anisotropic diffusion, the diffusivity along the direction of WM tracts, and the diffusivity perpendicular to the direction of WM tracts, respectively (Basser & Pierpaoli, 1996; Beaulieu, 2002). In particular, FA within a given WM voxel is presumably determined by the fiber diameter and density, degree of myelination, extracellular diffusion, interaxonal spacing, and intravoxel fiber tract coherence, whereas AD is generally related to axonal degeneration and RD is associated with the degree of myelination (Alexander, Lee, Lazar, & Field, 2007). Another frequently used diffusion parameter, mean diffusivity (MD), was not taken into account because it linearly depends on AD and RD. The three diffusion parameter images for each subject were nonlinearly normalized to the MNI space using the FMRIB linear image registration tool (FNIRT). A 6-mm Gaussian smoothing kernel was applied to the normalized images to compensate for the misalignment across individuals.

2.4.3 | Resting-state functional measures

Functional image preprocessing was performed using the DPABI toolbox (Yan, Wang, Zuo, & Zang, 2016). Briefly, the first 10 volumes were discarded to allow the magnetization to approach dynamic equilibrium and the participants to adapt to the scanner. The remaining volumes were then corrected for time offsets between slices due to interleaved acquisition and then realigned to the first volume to correct for inter-scan head motion. Individual anatomical T1 images were coregistered to the corresponding functional images and segmented into GM, WM, and CSF using SPM12. Subsequently, the normalized information derived from the T1 segmentation procedure was employed to normalize the resulting rs-fMRI scans to the standard MNI space, and then the images were resampled to a 3-mm isotropic resolution. Next, the normalized rs-fMRI images were linearly detrended and temporally bandpass filtered (0.01–0.1 Hz) to minimize the effects of low-frequency drift and high-frequency physiological noise. Several nuisance covariates were regressed out from the time course of each voxel, including head motion profiles (Friston 24-parameter model) and the global mean signal, WM signal, and CSF signal (Fox et al., 2005).

Here, we first measured the whole-brain functional connectivity strength (wFCS) at the voxel level. Pearson's correlation coefficients of the blood oxygen level-dependent (BOLD) time series between every pair of voxels within the GM mask were calculated and converted to Fisher's Z-values, which represent the strength of voxel pair-wise FC under the resting state. For a given voxel, its Z-values with every other voxel were summed up, which was defined as the

wFCS value of that voxel (Buckner et al., 2009; Tomasi & Volkow, 2010; Wang et al., 2014). Next, to quantify local spontaneous brain resting-state activity, we also computed the amplitude of low-frequency fluctuations (ALFF) (Zang et al., 2007).

2.5 | Statistical analysis

The chi-squared test was first employed to examine whether the karyotype (i.e., monosomy or non-monosomy) differed significantly between the two TS subgroups (the subnormal group: four girls with a monosomy karyotype; the abnormal group: four girls with a monosomy karyotype). Linear models were used to test the group differences in age or IQ scores. For the IQ scores, the karyotype (i.e., monosomy or non-monosomy) was included as a covariate.

GM and WM masks were first generated by thresholding the group-averaged GM and WM probability maps at 0.1, respectively. We assessed the effects of the "group-by-age" interaction on all brain parameters, GMV, WMV, FA, AD, RD, wFCS, and ALFF to evaluate whether hypogonadism influenced the age-related changes in adolescent girls with TS. Specifically, a linear model with "age", "group", and "group-by-age" as predictor variables was applied to each voxel (a) across the entire GM mask for the GMV and rs-fMRI measures (wFCS and ALFF), and (b) across the entire WM mask for the WMV, FA, AD, and RD, where the karyotype was included as a covariate to control for the potential confounding dosage effect of the X chromosome. For GMV and WMV, the total intracranial volume (TIV) was additionally included as a covariate in the model. For each brain measure, a Monte Carlo simulation method (i.e., 3dClustSim embedded in the AFNI) was applied to correct for multiple comparisons across voxels (Cox, 1996), and a family-wise error (FWE)-corrected *p*-value <.05 at the cluster level was considered significant.

For the remaining brain regions showing no significant effect of the "group-by-age" interaction, we further tested the main group effect after removing the "group-by-age" interaction term from the linear model while controlling for age, karyotype, and TIV (only for the GMV and WMV). The Monte Carlo simulation method of correcting for multiple voxel-wise comparisons was also applied (FWE-corrected $p < .05$). All the parametric statistical procedures were implemented using SurfStat (http://www.math.mcgill.ca/keith/surfstat/).

For the identified GM and WM clusters, we further tested whether these clusters were correlated with the IQ scores. For each pair of brain cluster and IQ score, we first tested whether the brain-IQ correlations differed between the two groups, that is, the "brain-by-group" interaction effects on IQ scores. Age and karyotype were also included as covariates. If there was no significant interaction effect, we further computed partial correlations between brain measures and IQ scores, while controlling for age, karyotype, and group factors.

3 | RESULTS

3.1 | Demographics and cognitive assessment

Demographics and cognitive assessment results are summarized in Table 1. No significant difference in age was observed between the two TS subgroups ($p = .22$). As expected, the abnormal group showed a significantly increased FSH level and decreased E2 level (FSH level: $p = 9 \times 10^{-6}$; E2 level: $p = .009$). Significant group effects were not observed for the five IQ scores, but the VCI ($p = .08$) and PRI ($p = .06$) showed a trend of group difference (Table 1). The χ^2 tests showed that both karyotype and pubertal status (Tanner stage) did not differ significantly between the two groups (karyotype: $\chi^2 = 0.61$, $p = .44$; breast: $\chi^2 = 3.11$, $p = .38$; pubic hair: $\chi^2 = 3.82$, $p = .28$). The two subgroups therefore could be considered as approximately matched in karyotype profile and pubertal status.

3.2 | Effects of hypogonadism on GM development during adolescence

As shown in Figure 1 and Table 2, only one cluster exhibited significant "group-by-age" effects on GMV ($F = 49.8$, $p = 2 \times 10^{-6}$), which was located around the medial orbitofrontal cortex (mOFC) and gyrus rectus in the left hemisphere. According to the post hoc analysis, the correlations between age and GMV in this cluster significantly differed

TABLE 1 Demographic characteristics and cognitive assessments

	Abnormal group ($n = 14$)	Subnormal group ($n = 9$)	Group effectt-value (*p*-value)
Age	14.30 ± 1.92	12.93 ± 3.27	1.27 (0.22)
FSH level (mIU/mL)	108.6 ± 35.87 (14)	6.76 ± 5.23 (9)	5.79 (9×10^{-6})***
E2 level (pmol/mL)	17.5 ± 15.25 (13)	64.73 ± 74.31(9)	2.90 (0.009)**
VCI	111.36 ± 17.78 (11)	99.43 ± 13.26 (7)	1.88 (0.08)
PRI	88.00 ± 9.52 (11)	77.14 ± 13.67 (7)	2.00 (0.06)
WMI	90.00 ± 15.26 (11)	87.71 ± 8.16 (7)	0.39 (0.70)
PSI	88.00 ± 13.75 (11)	84.14 ± 13.75 (7)	0.61 (0.54)
FSIQ	94.27 ± 13.69 (11)	84.43 ± 7.59 (7)	1.67 (0.12)

The parentheses after the cognitive scores represent the number of subjects who successfully performed the test. Abnormal group, patients with TS whose blood serum FSH level was greater than 40 mIU/mL; subnormal group, patients with TS whose blood serum FSH level was less than 40 mIU/mL.
Abbreviations: FSH, follicle-stimulating hormone; VCI, verbal comprehension index; PRI, perceptual reasoning index; WMI, working-memory index; PSI, processing speed index; FSIQ, full scale intelligence quotient.

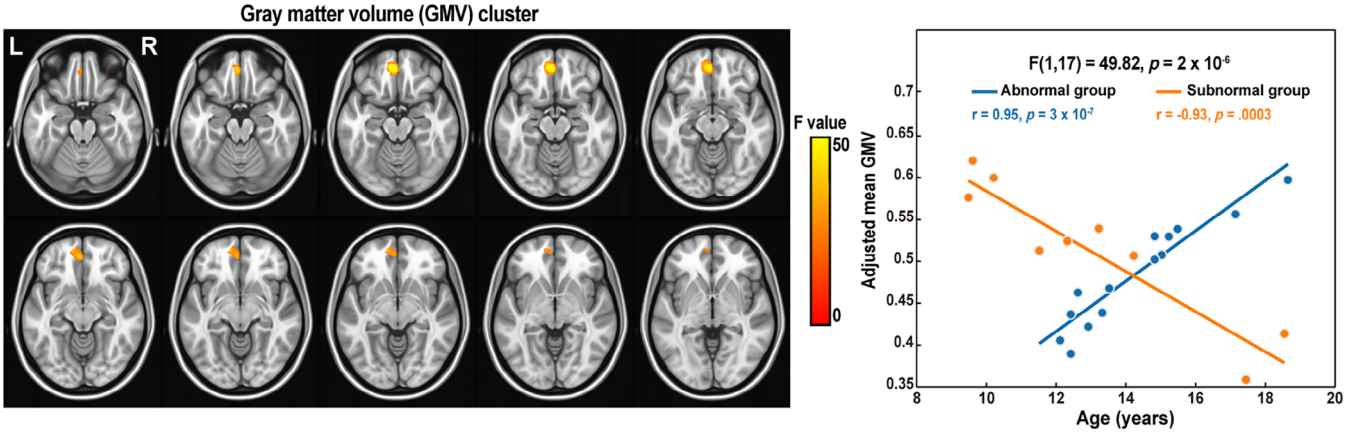

FIGURE 1 Significant effects of the interaction between age and group (abnormal group/subnormal group) on GMV. The GM cluster showing significant effects of the "group-by-age" interaction (FWE-corrected *p*-value <.05). The scatter plot on the right depicts the effects of the interaction between age and group on cluster-averaged GMV values for the significant cluster shown on the left. GMV, gray matter volume; FWE, family-wise error [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Clusters showing significant effect of the "group-by-age" interaction on GM or WM measures

Clusters	Anatomical regions	Volume (mm^3)	Peak F-value	MNI coordinates for peak voxel(x, y, z)		
GM measure						
Gray matter volume (GMV)						
1	Left medial orbitofrontal cortex Left gyrus rectus	2,251.13	88.07	−4.5	46.5	16.5
WM measures						
Axial diffusivity (AD)						
1	Left corticospinal tract Right corticospinal tract	5,528	18.49	−6	−12	−22
Radial diffusivity (RD)						
1	Left anterior thalamic radiation Right anterior thalamic radiation	10,656	21.57	12	−6	12
2	Left superior longitudinal fasciculus Left cingulum bundle	8,128	21.73	−34	−38	46
3	Left corticospinal tract Right corticospinal tract	6,504	15.00	−4	−36	−44

Peak coordinates were determined in the standard MNI space.

between TS subgroups: increased with age in the abnormal group (r = .95, $p = 3 \times 10^{-7}$) but decreased with age in the subnormal group (r = −0.93, p = .0003) (Figure 1). Regarding all the rs-fMRI measures, no significant "group-by-age" effect was observed across the GM mask.

For either GMV or rs-fMRI measures (i.e., wFCS and ALFF), we did not find significant clusters for the main group effect across the entire GM mask (for rs-fMRI measures) or the remaining GM regions (for GMV) after removing the "group-by-age" interaction term from the linear model above.

3.3 | Effects of hypogonadism on WM development during adolescence

The voxel-based analysis showed significant effects of the "group-by-age" interaction on WM diffusion measures. As shown in Figure 2 and Table 2, one significant AD cluster and three significant RD clusters were identified, but no significant FA or WMV cluster was observed. Specifically, a significant effect of the "group-by-age interaction on AD (F = 22.7, p = .0002) was observed in WM regions involving the left and right corticospinal tracts (Figure 2a). The largest RD cluster (F = 14.5, p = .001) was mainly located in the bilateral anterior thalamic radiations (Figure 2b), the second largest cluster (F = 15.4, p = .001) was located around the left superior longitudinal fasciculus and cingulum bundle (Figure 2c), and the last cluster (F = 19.9, p = .0003) was situated near the AD cluster within the left and right corticospinal tracts (Figure 2d). As illustrated in Figure 2, the "group-by-age" interaction exhibited a similar pattern for all these four significant WM clusters: the WM diffusion measures significantly decreased with increasing age in the abnormal group (post hoc: AD cluster, r = −.82, p = .0003; RD cluster 1, r = −.72, p = .004;

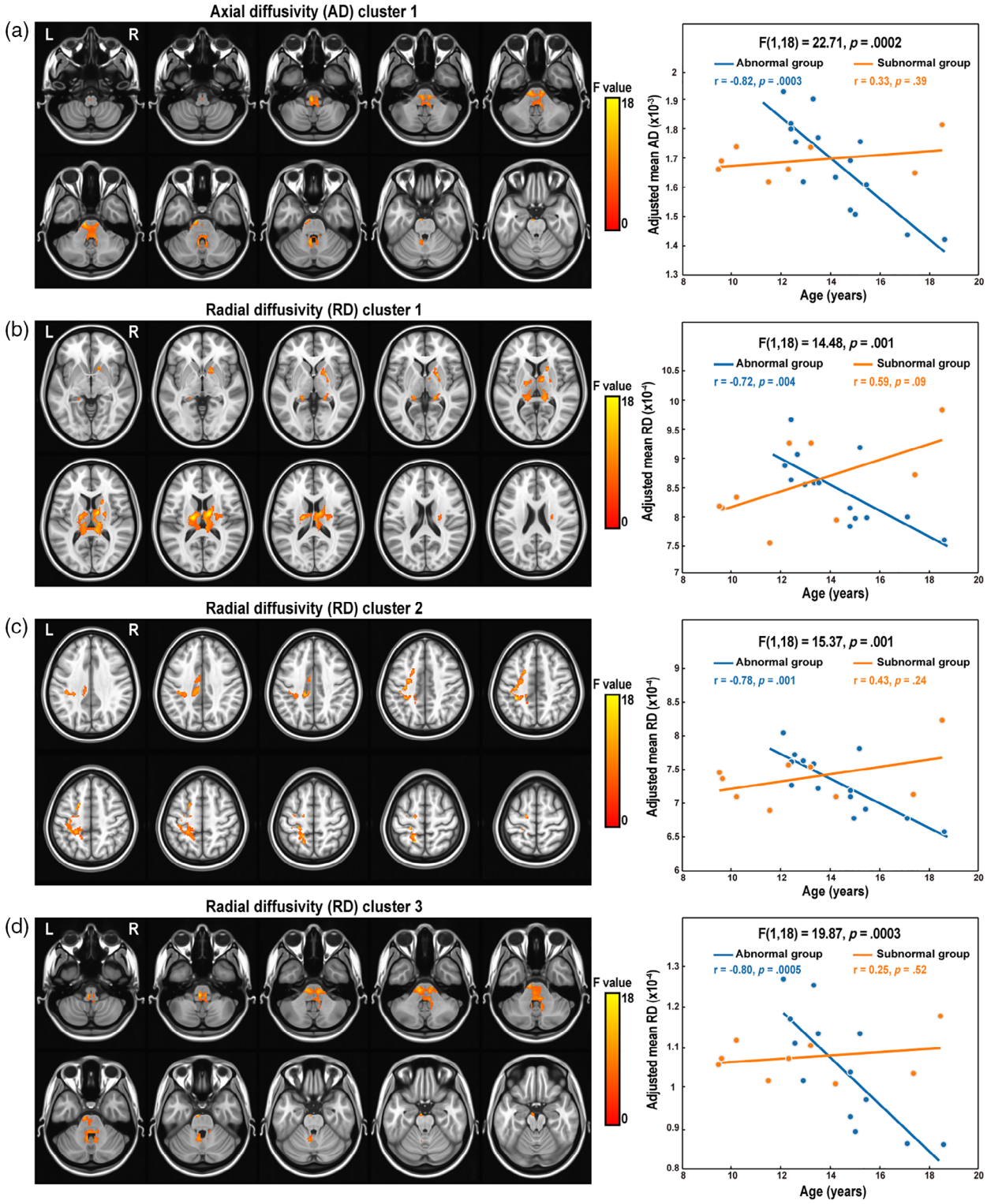

FIGURE 2 Significant effects of the interaction between age and group (abnormal group/subnormal group) on diffusion metrics. (a) One significant cluster for axial diffusivity (AD). (b–d) three significant clusters for radial diffusivity (RD). All clusters were considered significant if the FWE-corrected *p*-value was <.05 at the cluster level. The scatter plots shown in the right panels depict the effects of the interaction between age and group on cluster-averaged AD and RD values for these four clusters. FWE, family-wise error [Color figure can be viewed at wileyonlinelibrary.com]

RD cluster 2, $r = -.78$, $p = .001$; RD cluster 4, $r = -.80$, $p = .0005$), but were not significantly changed with increasing age in the subnormal group.

Again, for all these WM-relevant measures, we did not identify significant clusters for the main group effects across the entire WM mask (for FA and WMV) or the remaining WM regions (for RD and

AD) after removing the "group-by-age" interaction term from the linear model.

3.4 | Brain-IQ relationships

For all the identified GM and WM clusters above, we found neither significant "brain-by-group" interaction on any IQ score nor any significant brain-IQ score correlation.

3.5 | Validation

First, we re-estimated the statistical significance for the corresponding statistical models using a nonparametric permutation method by calling the "randomize" in FSL (Winkler, Ridgway, Webster, Smith, & Nichols, 2014). Significant clusters (FWE-corrected $p < .05$) were determined by the threshold-free cluster enhancement (TFCE) method. As shown in Figure S1, the statistical results from the nonparametric permutation method were quite consistent with our results above. Specifically, one similar GMV cluster showing significant "group-by-age" interaction was found around the mOFC. Regarding the AD and RD, while no cluster reached the significance level using this nonparametric permutation method, there was a strong trend towards significance for the afore-identified WM clusters (Figure S1b). Pearson correlation analysis was applied to quantify the spatial similarity between the significance maps. For all the three measures (GMV, AD, and RD), the significance maps from the nonparametric permutation method were highly similar with the ones from the parametric method (all $r = .99$), supporting the validity of our results above.

Given the continuous nature of the FSH, we next validated our current results by replacing the group factor with the FSH as a continuous variable in the corresponding statistical models. The same multiple comparison correction (i.e., the Monte Carlo simulation method) was applied. As shown in Figure S2, the statistical results for the "FSH-by-age" interaction effects were quite consistent with the above results for the "group-by-age" interaction effects. Specifically, there existed a significant "FSH-by-age" interaction effect on GMV in a cluster around the mOFC. Regarding AD and RD, no cluster reached the significance level for the "FSH-by-age" interaction effect after multiple comparison correction, but there was a strong trend towards significance for the afore-identified WM clusters (Figure S2b). Likewise, for all the three measures (GMV, AD, and RD), the significance maps for the "group-by-age" interaction effect were highly similar with the ones for the "FSH-by-age" interaction effects (r range: 0.81∼0.87), suggesting robustness of our current results.

4 | DISCUSSION

By comparing adolescent girls with TS presenting with severe and mild hypogonadism, the present study revealed a significant effect of hypogonadism on both GM and WM development, suggesting an important contribution of ovarian hormones especially the estrogen to neurodevelopment during adolescence under the condition of X chromosome insufficiency.

While many neuroimaging studies have assessed the neuroanatomical and neurofunctional differences between girls with TS and healthy controls, these studies largely suffered from the limitation of the difficulty in differentiating the direct genetic effect from the indirect hormonal effects based on their results. To our knowledge, the present investigation represents the first neuroimaging study that explored the pure effects of hormone levels on brain development after controlling for the genetic effect within the TS population. Therefore, our findings are of particular importance for understanding the roles of ovarian hormones in brain development during adolescence in patients with TS.

Girls with hypogonadism do not exhibit a surge of estradiol release from the gonads through the reactivation of the hypothalamic–pituitary-gonadal axis, which spontaneously occurs in healthy girls. In these girls, decreased estrogen levels together with the feedback mechanism lead to an elevated level of FSH. Therefore, elevated FSH production has been deemed as a key endocrinological indicator of estrogen deficiency. Accordingly, the present study categorized the girls with TS using a recommended FSH cutoff (i.e., 40 mIU/mL), providing an opportunity to intuitively determine the impact of hypogonadism on brain development in adolescent girls with TS.

Animal studies have consistently shown that gonadal steroids influence brain organization via multiple neurodevelopmental processes, including neurogenesis, and neurite outgrowth (McEwen & Alves, 1999), axon myelination (Yates & Juraska, 2008) and the growth of astrocytic processes (Chowen, Azcoitia, Cardona-Gomez, & Garcia-Segura, 2000). In humans, higher estradiol levels are associated with a decreased GMV (girls only, [Peper et al., 2009]; both sexes combined, [Herting et al., 2014]), smaller anterior cingulate cortex and amygdala (Koolschijn, Peper, & Crone, 2014), and larger parahippocampal regions (sexes combined; [Neufang et al., 2009]). During healthy puberty, GMV in the frontal lobe exhibits an inverted U-shaped trajectory in which a pre-pubertal increase is followed by post-pubertal loss (Giedd et al., 1999). Frontal GMV peaks approximately 1 year earlier in females than males, which may be a consequence of the earlier age of onset of puberty in females and suggests a possible influence of gonadal hormones (Giedd et al., 1999). By comparing TS girls with healthy controls, Lepage and colleagues revealed aberrant neurodevelopmental trajectories in TS, relative to controls (Lepage, Mazaika, Hong, Raman, & Reiss, 2013). While it is not possible to disentangle the effect of estrogen deficiency from the direct genetic effect of X-monosomy by just making a comparison between TS and healthy controls, the authors argued that their finding may, at least, in part be attributed to the pre- and postnatal estrogen deficiency in girls with TS. Along this line, our current study stepped further and compared two TS subgroups with approximately matched karyotypes but different degrees of hypogonadism/estrogen deficiency. The results revealed that the age-related changes of GMV around the left mOFC showed a hypogonadism degree-dependent manner within the TS population. Specifically, GMV of the left mOFC decreased in the subnormal group, which roughly followed a "near-typical" maturational

reduction. In contrast, GMV of the left mOFC in the abnormal group increased significantly as a function of age, indicating aberrant developmental changes in TS girls with severe hypogonadism. Together with previous results in healthy, our findings highlight a critical hormonal role in brain development for both healthy and TS.

Notably, the OFC is a prefrontal cortex region that is strongly involved in the cognitive processing of emotion and reward during decision-making (Bechara, Damasio, & Damasio, 2000). Impaired social cognitive processing and executive function deficits have been repeatedly reported (Wolstencroft & Skuse, 2019). The currently observed "off-track" orbitofrontal changes during adolescence in girls with TS presenting with severe hypogonadism therefore might relate to the disrupted social cognition in these patients, highlighting a substantial role for estrogen in this area and social cognition.

Similarly, the age-related changes of specific WM tracts differed between girls with TS presenting with severe and mild hypogonadism, including the corticospinal tract, thalamic radiation, superior longitudinal fasciculus, and cingulum, as revealed by the diffusion measures (i.e., AD and RD). In particular, diffusion measures across all these clusters were consistently decreased as a function of age in the girls with TS presenting with severe hypogonadism but not in the girls with TS presenting with mild hypogonadism, suggesting a similar effect of hypogonadism/estrogen deficiency on these tracts. In healthy brain development during adolescence, most individuals exhibit little or no change with age for both RD and AD measures in association WM fibers including cingulum, corticospinal tract and superior and inferior longitudinal fasciculus (Lebel & Beaulieu, 2011). These findings were compatible with our currently observed subtle age-related RD changes in thalamus radiation and cingulum in the subnormal group, suggesting "near-typical" age-related changes of these association fibers in TS girls under relatively-reserved ovarian function.

Interestingly, our currently observed GM clusters around the mOFC and WM clusters around the thalamus radiation and cingulum are both located within the limbic network, which is a key target of ovarian sex hormones (Braun, 2011; Catenaccio, Mu, & Lipton, 2016). Particularly, the observed "group-by-age" interaction effects of the GM and WM clusters are very convergent in the two TS groups: the subnormal group exhibited a "near-typical" development, while the abnormal group had an "off-track" developmental change. These findings together suggested an important modulating role of ovarian function and estrogen in the neurodevelopment of the limbic network during adolescence.

Additionally, both AD and RD clusters round the corticospinal tract showed significant differed developmental changes between TS groups. These diffusivity measures in corticospinal tract decreased with age in the abnormal group but remained unchanged with age in the subnormal group. The corticospinal tract primarily involves the primary motor cortex and is associated with the motor control of the body and limbs (Guyton & Hall, 2006). In particular, estrogen-treated girls with TS show improved motor speed than placebo-treated girls with TS (Ross, Roeltgen, Feuillan, Kushner, & Cutler Jr., 1998), suggesting an effect of estrogen on the corticospinal tract. The abnormalities in the pubertal age-related changes of diffusion measures in the corticospinal tract observed in the present study might be partly responsible for the motor deficits in patients with TS during puberty.

Notably, the rs-fMRI measures (i.e., wFCS and ALFF) showed neither significant "group-by-age" interaction effect nor between-group difference, suggesting a tenuous or unstable effect of hypogonadism/estrogen deficiency on brain functional activity during resting state in TS girls. Although the limited statistical power due to our small sample size may account for these negative results, there are compatible findings. For example, there was no significant difference in wFCS between different menstrual phases in healthy females (Syan et al., 2017). Interestingly, the only two rs-fMRI studies in TS consistently revealed reduced wFCS in the bilateral intraparietal sulcus and cerebellar regions, relative to healthy controls (Green, Saggar, Ishak, Hong, & Reiss, 2018; Xie et al., 2017). Together with our currently observed negative results, the observed wFCS reduction in TS relative to healthy controls is likely associated with the direct genetic effect, rather than the indirect hormonal effect induced by the X chromosome deficiency.

GH deficiency is present in some girls with TS. Previous studies have shown a nontrivial effect of GH deficiency on brain structures (Annenkov, 2009; Deijen, Arwert, & Drent, 2011; Webb et al., 2012). In girls with TS with concurrent deficiencies in both GH and estrogen, abnormalities in brain development during adolescence are likely associated with the lack of both hormones. In the present study, all girls with TS except one underwent GH substitution, and only four girls were on ER treatment. Therefore, our current findings are largely attributed to the estrogen deficiency, rather than the GH deficiency. It is worth mentioning that the relative absence of ER therapy in the present study is likely attributed to current clinical practice regarding the timing of ER therapy, as well as the late diagnosis of TS patients. To increase the final adult height of girls with TS, postponing ER therapy until the mid-teens has been commonly recommended because estrogen/puberty accelerates epiphyseal fusion and thereby reduces adult height (Chernausek, Attie, Cara, Rosenfeld, & Frane, 2000; Saenger et al., 2001; Tanner, Whitehouse, Hughes, & Carter, 1976). This clinical practice of postponing ER therapy together with the relatively late TS diagnosis in the present study leaded to a delayed application of ER therapy for our recruited TS girls.

Regarding the IQ scores, near-significant group effects were observed for the VCI ($p = .08$) and PRI ($p = .06$) scores, with the abnormal group slightly higher than the subnormal group. The direction of the difference is counterintuitive, which also conflicts with the previously reported improvement of cognitive performance after estrogen-treatment in TS (Rovet, 2004). The interpretation for this unexpected result is difficult by using our current data. Future studies with larger sample size and more detailed cognitive tests are warranted to verify these results and provide more data for interpretation. In addition, the present study did not find any significant brain-IQ correlation, which might relate to the limited cognitive specificity of IQ scores. To better understand the hormone-brain-cognition pathway, specific social/emotional cognitions that are associated with the limbic system need to be comprehensively evaluated in the context of the brain-cognition relationship in the future.

Finally, several limitations of our present study should be addressed. First, the sample size was quite small due to the small number of patients with TS, and therefore the statistical power was limited. This might account for some negative results, for example, no difference in rs-fMRI measures in patients with TS. Given the limited statistical power, while the voxel-wise multiple comparisons were corrected for each brain measure, we did not correct for multiple comparisons for the level of brain measures. Therefore, the statistical results (e.g., the identified significant clusters) should be taken as exploratory but not confirmatory. Next, the two TS groups in the present study included girls with X monosomy, mosaicism, or other types of complex X-linked karyotype abnormalities. When roughly classifying the karyotype into monosomy or non-monosomy, the two groups showed an approximate match in this karyotype variable, and we further included this karyotype variable as a covariate in the analyses. However, this scheme is limited by over-simplifying the karyotype, and the mixed complex karyotypes did exist in both groups, which may confound our results. Future investigations using TS girls with homogeneous karyotypes are highly desired to validation our current findings. In addition, other gonadal hormones (e.g., the estrogens that are locally synthesized from neurosteroids and testosterone) may also influence the brain maturation process as well (Bramen et al., 2011; Colciago, Casati, Negri-Cesi, & Celotti, 2015; Pelletier, 2010) but were not controlled in the present study. Lastly, the current study only included two subgroups of girls with TS to evaluate the effects of hypogonadism/estrogen deficiency within the TS population. In future work, girls without TS can be included as a good control for specific investigations. For instance, a group of girls with normal genetic profiles but suffering from ovarian dysfunction will make it possible to assess the direct X-linked genetic influences by matching the ovarian condition with TS girls.

5 | CONCLUSIONS

During adolescence, girls with TS presenting with mild and severe hypogonadism exhibited differential neurodevelopmental patterns during adolescence for the GMV and WM microstructure in specific brain regions. These findings provide insights into how estrogen deficiency impacts brain development in adolescent patients with TS, and highlight an important role of gonadal hormones in the brain and cognition in general.

ACKNOWLEDGMENTS

This work was supported by the National Science Foundation of China (No. 91732101, 81671772, 81701783), the Research Fund of PLA of China (AWS17J011), and the Fundamental Research Funds for the Central Universities.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

CONFLICT OF INTERESTS

The authors declare no potential conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Li M, Zhao C, Xie S, et al. Effects of hypogonadism on brain development during adolescence in girls with Turner syndrome. *Hum Brain Mapp*. 2019;40: 4901–4911. https://doi.org/10.1002/hbm.24745